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## Note

### Improved N-chlorination procedure for detecting amides, amines, and related compounds on thin-layer chromatograms

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In connection with a study on the isolation of drugs containing an amide group from the tissue of farm animals, we have developed and describe below a simple, sensitive procedure for detecting these and some other biologically important classes of nitrogenous compounds on thin-layer chromatograms. The method involves the N-chlorination of a primary or secondary amide or amino group with chlorine vapor evolving from the slow, spontaneous decomposition of calcium hypochlorite, followed by selective reduction of the excess chlorine with formaldehyde vapor. The N-chloro compounds are then detected as purplish-blue spots by spraying with potassium iodide–starch solution containing a wetting agent. Although there are several N-chlorinating procedures available<sup>1–6</sup>, the proposed procedure is faster and more convenient.

#### EXPERIMENTAL\*

All chemicals were from J. T. Baker (Phillipsburg, NJ, U.S.A.). Thin-layer plates were products of Analtech (Newark, DE, U.S.A.); Whatman (Clifton, NJ, U.S.A.); and E. Merck (Darmstadt, G.F.R.). Silica gel plates 2.5 × 10 cm and 20 × 20 cm, with and without fluorescent indicator, and coated with a 250-μm-thick layer were employed.

The plates were spotted with nanogram amounts of the compounds and developed with a solvent which moved the spots above the origin but behind the solvent front. They were dried at 100°C for 5–10 min unless there were compounds on the plate that volatilized at this temperature, in which case they were dried at room temperature for 15–60 min, or overnight. The plate (cooled to room temperature, if heated) was placed for 2 min in a tank containing an approximate 0.5-cm layer of calcium hypochlorite (renewed every 6–7 days) spread over the bottom. It was then transferred to a tank containing a beaker of formalin (renewed every 6–7 days) (20 ml for a tank 30 × 27.5 × 8.7 cm) for 30–45 sec, removed, and sprayed with a solution containing 1% soluble potato starch (dissolved by heating), 1% potassium iodide, and 0.05% Triton X-100.

\* Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

## RESULTS AND DISCUSSION

The compounds studied, developing solvents,  $R_F$  values, and the minimum amount detectable by the procedure after chromatographic development are given in Table I. Animal drugs, lipids, nucleotide bases, amino acids, peptides, and a few miscellaneous compounds are represented in Table I. Several compounds did not give a positive response at relatively high concentration. Diphenylamine, 2,4-dinitrobenzamide (nitromide) and methionine sulfoxide gave no response at the 1–2- $\mu$ g level.

Reproduction of two chromatoplates showing the expected contrast between spots and background is shown in Fig. 1.

TABLE I

### LOWER LIMIT OF DETECTION OF COMPOUNDS BY N-CHLORINATION PROCEDURE

Solvent systems: a = Ethyl acetate; b = ethyl acetate-methanol-water (68.5:26.5:5); c = methanol; d = water-methanol (2:1); e = methylene chloride.  $R_F$  values were determined on Analtech plates. Minimum detectable amounts were determined on Analtech plates,  $2.5 \times 10$  cm.

Compound	Solvent system	$R_F$	Minimum detectable amount (ng)
Chloramphenicol	a	0.61	60
Aklomide	a	0.64	50
Zoalene	a	0.79	57
Sulfantran	a	0.78	46
Nitrofurazone	a	0.28	136
Anthranilic acid	a	0.22	43
Methyl anthranilate	e	0.67	37
<i>p</i> -Aminobenzoic acid	a	0.30	38
Sulfanilic acid	b	0.29	32
Creatine	c	0.26	28
Cytosine	c	0.52	13
Inosine	c	0.61	77
5-Methylcytosine	c	0.52	10
Adenine	c	0.64	10
Guanine	c	0.65	11
Xanthine	c	0.71	20
Thymine	c	0.81	23
Uracil	c	0.81	23
Hypoxanthine	c	0.70	10
Histidine · HCl	d	0.47	17
DL-Phenylalanine	c	0.09	66
Glycylglycine	d	0.82	33
Glycylglycylglycine	d	0.82	33
O-Phosphoethanolamine	d	0.54	29
Sphingosine	a	0.72	50
DL-Phosphatidylethanolamine	b	0.20	29
dipalmitoyl glycerol			
Octadecylamine	b	0.14	31
$\alpha,\alpha$ -Dimethylphenethylamine	b	0.12	30
Dicyclohexylamine	b	0.14	33

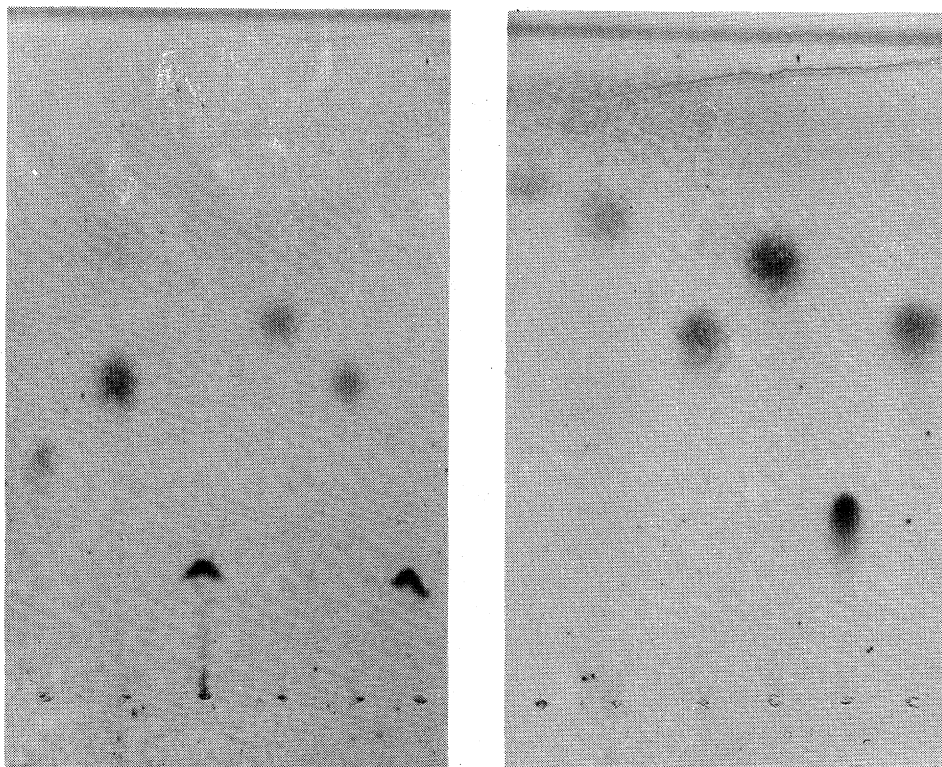


Fig. 1. Showing spots after N-chlorination procedure. Right chromatoplate: compounds from left to right are thymine (46 ng), xanthine (40 ng), 5-methylcytosine (20 ng), guanine (22 ng), creatine (56 ng), and cytosine (26 ng). Analtech silica gel G  $2.5 \times 10$  cm; developing solvent, methanol. Left chromatoplate: compounds from left to right are chloramphenicol (120 ng), sulfanitran (92 ng), anthranilic acid (86 ng), zoalene (114 ng), aklomide (100 ng), and *p*-aminobenzoic acid (76 ng). Analtech silica gel G  $2.5 \times 10$  cm; developing solvent, ethyl acetate.

Developing systems containing ammonia or nitrogen-containing organic solvents can give high background color and should be avoided unless sufficient time is allotted for their complete volatilization from the plate prior to N-chlorination.

Silica gel layers incorporating a fluorescent indicator were less amenable to the detection of the compounds studied than were normal plates, requiring about twice the minimum detectable levels listed in Table I.

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